ERDC/CERL TR-02-34

Construction Engineering Research Laboratory

US Army Corps of Engineers®
Engineer Research and Development Center

Effects of Fog Oil Smoke on the Hatchability and Fledgling Survival of the House Sparrow (*Passer domesticus*), a Nestling Surrogate for the Red-cockaded Woodpecker

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December 2002

Foreword

This study was conducted for the Strategic Environmental Research and Development Program (SERDP) under project number CS-507, "Threatened, Endangered, and Sensitive Resources: Impact of Smokes and Obscurants on TES." The technical monitor for the project was Dr. Femi A. Ayorinde, SERDP Cleanup and Conservation Program Manager, followed by Dr. Robert W. Holst, Compliance and Conservation Program Manager. The Executive Director of SERDP is Mr. Bradley P. Smith.

The work was completed under the direction of the Ecological Process Branch (CN-N) of the Installations Division (CN), Construction Engineering Research Laboratory (CERL). The CERL Principal Investigator and contract monitor was Dr. Keturah Reinbold. The work was performed by the Pacific Northwest National Laboratory (PNNL), Richland, Washington. Crystal Driver was the PNNL Principal Investigator. Jennifer Ollero, Yin Fong Su, Robert Fulton, Gary Dennis, and Brett Tiller are also employed by PNNL. The work was completed under Military Interdepartmental Purchase Requests (MIPRs) W52EU261647000, W52EU28254173, and W2V5AA51305044. The technical editor was Gloria J. Wienke, Information Technology Laboratory. Dr. Harold Balbach is the Threatened and Endangered Species Research Project Leader. Steve Hodapp is Chief, CEERD-CN-N, and Dr. John T. Bandy is Chief, CEERD-CN. The associated Technical Director was Dr. William D. Severinghaus, CEERD-CV-T. The Director of CERL is Dr. Alan W. Moore.

The authors thank Bruce Hagedorn, "Jackson Guard," Natural Resources Branch, Eglin Air Force Base, Florida, for supplying the red-cockaded woodpecker cavities. Special thanks also go to Patricia M. Kirby, Colorado State University contractor, who coordinated preparation of the final report.

CERL is an element of the U.S. Army Engineer Research and Development Center (ERDC), U.S. Army Corps of Engineers. The Commander and Executive Director of ERDC is COL John Morris III, EN and the Director of ERDC is Dr. James R. Houston.

Pacific Northwest National Laboratory
Operated by Battelle for the U.S. Department of Energy

Prepared for the U.S. Department of Energy under a related services contract for the Engineer Research and Development Center/Construction Engineering Research Laboratory under contract DE-AC06-76RL01830.

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1 Introduction

Background

Domestic Army installations, when combined with installations of other U.S. military services, total more than 25 million acres (about 10 million hectares). Among this land area are significant parcels where the intensity of use is low enough, or infrequent enough, to support populations of species considered rare outside the installation. When the aggregate area of essential or preferred habitat is adequate to support their population, threatened and endangered wildlife species often find refuge on military lands. Therefore, the essential military functions occurring on military bases must coincide with efforts ensuring the continued health of the critical populations of these species, while maintaining the operational status of the installations. One such challenge falls under the Endangered Species Act of 1973 (Public Law 93-205; 16 U. S. Code 1531 et seq., as amended), which mandates the maintenance/expansion of red-cockaded woodpecker (***Picoides borealis***) populations in areas of troop readiness training.

Among the training activities that occur in or near woodpecker habitat is the generation of aerosols of obscurant materiel. Obscurants have long been used to mask movements of troops and mechanized equipment. Of the conventional smokes, the white smoke generated from vaporization and condensation of liquid fog oil (SGF-2) is an effective obscurant in the visible range. It is the most heavily used obscurant for troop training because of its low cost, ease of handling and smoke generation, dispersion characteristics, and safety (Eberhard, Cupp, and Abshire 1989).

It must be noted that the term “fog oil smoke” is somewhat misleading. While the white clouds emitted from the generators are commonly called “smoke,” they really are better described as “fog,” and are composed of small droplets, not unlike a fog composed of water droplets. Ideally, no combustion products are present in this fog, and the oil is not “burned” by any process. In practice, however, the heated portions of the generator may cause thermal recombination in a small portion of the fog oil, and the engine powering the military generating equipment may contribute exhaust products.

Troop preparedness training and the field testing of generating equipment often involves release of fog oil into the atmosphere. Managing sensitive avian populations, in conjunction with providing realistic troop training opportunities, requires an accurate assessment of the toxicity and health effects of fog oil to species such as the red-cockaded woodpecker. However, the effects of airborne fog oil on wildlife and, in particular, avian wildlife are poorly known or unknown. It is important to determine potential adverse effects in birds because they are often more sensitive to airborne pollutants than mammals, they have high public visibility, and they are used as bio-indicators of ecosystem health.

Prior to the conduct of the studies completed for the Army and reported through technical reports for the Engineer Research and Development Center-Construction Engineering Research Laboratory (ERDC-CERL) (Driver et al 2002 and Nam et al 1999), the toxic effects of airborne fog oil aerosols on wild birds could only be inferred from (predominately marine) oil-spills and from laboratory studies simulating oral and dermal exposures from such spills (Hartung and Hunt 1966, Hartung 1967, Holmes and Cronshaw 1977, Miller et al. 1978a and 1978b, Leighton 1982, Leighton et al. 1985). These data are often conflicting due to significant differences in petroleum composition, dosing regime, bird species, and age class tested (Clark 1984). Virtually no data were previously available on the inhalation toxicity of petroleum-based oils or polynuclear aromatic hydrocarbons (PAHs, the more toxic components of petroleum oils) in birds. Inhalation of fog oil aerosols has been investigated in human surrogate species (for example, laboratory rodents) and harmful exposure concentrations extrapolated for humans. However, the application of these values to the wide variety of wild animal species is problematic because both the sensitivity to air pollutants and the doses received (on a body-weight basis) differ greatly among species (Hill 1994; Schafer 1972; Schafer, Bowles, and Hurlbut 1983). For example, the volume of air breathed per minute per unit of body weight (the weight-specific minute ventilation) varies greatly among mammals (Phalen 1984). Generally, the smaller the animal, the more air per minute per gram is inhaled. Compared to humans, rabbits ventilate 3 times the volume of air and small rodents ventilate 8 to 13 times the volume, on a per-body-weight basis (Phalen 1984). Birds may be at even greater risk of exposure because their respiratory rates are generally higher than those of mammals of comparable size. However, because of fundamental differences in their respiratory systems, mammalian inhalation data cannot be extrapolated to avian species. Similarly, feather deposition and subsequent oral uptake via preening cannot be estimated from mammalian studies. For bird species with critical populations (threatened and endangered species), exposure and toxicity are best estimated from size-specific surrogates. The results of such a study on a surrogate for the red-cockaded woodpecker (RCW) were reported in a previous report in this series (Driver et al. 2002).

From the available oil-spill studies, the susceptibility of birds to the toxic effects of petroleum from oral and dermal exposure appears to be much greater than that of many other organisms (Geraci and Smith 1977; Grau et al. 1977; Hartung and Hunt 1966; Hartung 1967; Chia 1971; and Rowe, Dollahite, and Camp 1973) and refined oils are more toxic to birds than are crude oils (Fleming, Sileo, and Franson 1982). This inordinate susceptibility probably results from the ability of feathers to readily absorb large quantities of oil immediately upon contact. Absorption of the oil by the feathers retains oil for ingestion through preening (and contamination of eggs in nesting birds) and deprives the birds of the critical functions of their feathers (such as insulation, water repellency, and flight). The impact of oil aerosols, such as SGF-2 obscurant, on feather function and subsequent health deficits has not been investigated. However, given the absorption potential and sensitivity of feathers to oil contamination, such studies are necessary for accurate hazard assessments. The present study carries this investigation to effects on hatchlings and nestlings as opposed to exposure of adult birds.

SGF-2 fog oil (FO) is the obscurant most often used for military training. It is a middle distillate product of crude petroleum and is drawn from raw industrial lubricant oil. FO procured by the U. S. military has undergone a modified refining process to reduce quantities of potentially harmful components. Although called a "smoke" because of its appearance when generated in the field, it is not burned, but rather vaporized and disseminated by re-condensation as the vapors cool in the air. Airborne FO droplets have a mass median aerodynamic diameter (MMAD) typically between 0.9 and 1.9 μm (Driver et al. 1993), a size range that deposits within the lung and air sacs of birds (Driver et al. 1990).

Most of the information on avian response to petroleum oil contamination is derived from marine oil spills and from laboratory studies simulating oral and dermal exposures from aquatic spills (Hartung and Hunt 1966, Hartung 1967, Holmes and Cronshaw 1977, Miller et al. 1978a and 1978b, Leighton 1982, Leighton et al. 1985). Little data are available on inhalation toxicity of petroleum-based oils in birds. Only one study has investigated the acute health impacts of respiratory exposure to FO in wild captive birds. In this study, described in Driver et al. 2002, adults of a surrogate species (the red-winged blackbird, ***Agelaius phoeniceus***) having a size-adjusted minute volume similar to the red-cockaded woodpecker were exposed to field typical (100 mg/m^3) and higher (400 mg/m^3) airborne concentrations of FO for 4 hours. These concentrations were tolerated without adverse effects. Deposition of FO to feathers of the birds exposed to obscurant was shown (Driver et al. 2002); however, the amount deposited and potentially ingested through preening was below harmful levels.

The amount of FO deposited to the feathers was also below hypothermic thresh-

old doses for petroleum oil (Hartung 1967, Driver et al. 2002), and no impact of FO deposition on feather function (thermal insulation, water repellency, flight) and subsequent body weight and carcass condition was observed.

Although FO dissemination appears to have little acute impact on wild adult birds, the effects of fog oil exposure on younger age classes cannot be extrapolated from these data, particularly for the naked young of altricial species. Eggs are also a potentially sensitive stage for FO exposure. Application of minute amounts of lubricating oils (No. 2 fuel oil) to egg surfaces is highly toxic to embryos of aquatic birds (Albers 1977; Szaro and Albers 1977, White et al. 1979).

Objectives

The objectives of this series of studies were to evaluate the health effects of fog oil aerosols in various species surrogates for the red-cockaded woodpecker, and to provide information for general and site-specific risk assessments and the management of obscurant fog oil generations and training activities. The objective of the current study was to evaluate the effect of field typical dissemination of FO obscurant on (1) the hatchability of eggs and the survivability of nestlings and fledges produced from eggs exposed to FO aerosol during early embryo development, and (2) the fledging success of nestlings exposed to FO during their first week of development. Realistic exposure simulation for cavity-nesting wild birds such as the red-cockaded woodpecker was emphasized.

Scope

The studies described in this report are limited to examination and discussion of the effect of fog oil smoke on eggs, hatchlings, and nestlings of surrogate avian species, as well as examination of the survivability of the fledglings as adults, and of their reproductive success, and examination of possible abnormalities in successive generations.

Approach

The avian species of direct concern is, as stated earlier, the red-cockaded woodpecker (***Picoides borealis***). Since this is a listed species, and because no other roughly similar woodpecker species was available in populations adequate to conduct the proposed studies, a species surrogate was selected for these studies. The house sparrow (***Passer domesticus***) was used as the test species in this por-

tion of the study because it is sensitive to environmental pollutants (Tucker and Haegele 1971, Schafer et al. 1983, Hill 1971, Schafer 1972, Schafer et al. 1973) and, because of its small size, has weight-specific ventilation that results in a relatively high inhaled dose of airborne contaminants (Phalen 1984). It was also selected for this phase of the study because it will nest in cavities with dimensions similar to cavities excavated by the red-cockaded woodpecker and because its semi-colonial nesting habit allows for a large number of active nests within a small area. Also, it is an altricial species, like the red-cockaded woodpecker; the hatchlings have little to no down at hatching and increased potential for dermal exposure to aerosols compared to common precocial test species.

To ensure the artificial nest boxes used in this study provided a fog oil exposure to the eggs and nestlings similar to that which occurs in natural nest cavities, the internal dimensions of the nest box cavities were altered to simulate the internal conformation of naturally excavated cavities. To accomplish this, natural red-cockaded woodpecker nest cavities were cut from wind-felled trees (Figure 1) obtained from Eglin Air Force Base, Niceville, Florida.

Figure 1. Three wind-felled nest cavities of the red-cockaded woodpecker used to design internal volumes of the test nest boxes and to determine the relationship between external and internal concentration profiles of airborne fog oil during smoke generation.

An exposure chamber into which a known concentration of FO could be generated and maintained during the course of the exposure was used to deliver the test exposures of FO. Clutches of eggs or broods of nestlings were exposed in their nest boxes during the first 5 days of their development. Based on studies

that modeled concentrations of fog oil under typical field use conditions, two concentrations of fog oil were used in the exposure tests: a typical field concentration (100 mg/m^3) and a high, near-source concentration of 450 mg/m^3. The nest boxes were exposed for 30 minutes at the treatment concentration. In addition, the nest boxes were exposed to an additional 15 minutes of aerosols of lower concentration as the FO concentration built up to the target concentrations of the test atmospheres within the exposure chamber.

FO deposition on eggs was further estimated by placing Northern Bobwhite (***Colinus virginianus***) eggs in a nest box and exposing the box to FO as described for the test exposures. Eggs were also placed on the floor of the exposure chamber outside of the nest box. Foil deposition coupons were placed on the nest box and on the floor of the chamber next to the eggs that were placed outside the nest box.

Mode of Technology Transfer

The information included in this report is one portion of the materials prepared by the Engineer Research and Development Center (ERDC) to assist installation natural resources and threatened and endangered species program managers. The primary means of communicating the hatchling and nestling toxicity information will be through publication in the scientific literature, as well as through the availability of this report. The specific data presented are intended to be used in the preparation of biological opinions related to planned Army actions where the red-cockaded woodpecker (or other similar avian species) is present, and for endangered species management plans (ESMPs), integrated natural resources management plans (INRMPs), and in the preparation of ecological risk assessments involving fog oil smoke and avian species.

This report will be made accessible through the World Wide Web (WWW) at URL: http://www.cecer.army.mil

2 Methods

Test Animals

Wild adult house sparrows were baited to traps in Benton and Franklin Counties, Washington, and transferred to an outdoor aviary at Pacific Northwest National Laboratory (PNNL). A total of 220 (100 male and 120 female) adult house sparrows were captured in late fall and maintained over the winter for nesting in spring.

One-third of the 9.1-m wide by 15.2-m long by 3.7-m high (30 ft by 50 ft by 12 ft) aviary was covered with a metal roof. Wooden rods suspended from the roof frame provided perches for the birds. Roosts consisted of fir (***Abies*** spp.) and spruce (***Picea*** spp.) trees placed in planters and arranged around 3.3-m (4 ft) high omni-directional roost boxes. Willow trees (***Salix*** spp.), spruce trees, arborvitae (***Thuja occidentalis***), pine (***Pinus*** sp.) and a variety of dwarf conifers and rhododendron provided natural cover in the open areas of the aviary. Continuous flowing water was provided in five 3-m long troughs. Five dusting trays were also provided and the sand changed every 2 days.

Birds were fed a pelleted, complete diet for insect eating soft-billed birds (Product No. 73534800, Zeigler Bros, Inc., Gardners, PA). Although the diet was complete, cracked corn, wheat, natural millet sprays, commercial mix of wild birdseed, berries, and lettuce were also provided ***ad libitum*** for both dietary variety and environmental enrichment. These foods were distributed in 30 feeding stations at various heights throughout the aviary. In mid-February, an additional 40 stations were added to distribute mealworms (***Tenebrio molator*** in three size-classes, and ***Zophobas morio***). When nest building was initiated, larvae of the greater bee moth (***Galleria mellonella***) and fresh corn (on the cob) were added to the mealworm feeding stations. All larvae were obtained from Grubco (Hamilton, Ohio). Three clear Plexiglas® open-topped boxes (60 cm 60 cm x 60 cm) were used to house mixtures of three size-classes of crickets (***Acheta domestica***). To maintain the food value of the larvae and crickets, oatmeal and commercial cricket food (Ghann's Cricket Farm, Inc., Augusta, Georgia), fresh corn, and slices of potato and/or carrot were provided daily in each feeding station. Wood shavings were used to provide cover for the larvae. During high consumption periods (when nestlings hatched), larva and cricket stations were restocked a

minimum of 4 times per day. Two commercial automatic directional feedcasters (Moultrie Feeders Inc., Alabaster, Alabama) were elevated to 1 and 2 meters above the aviary floor to broadcast mealworms co-mixed with seed every 2 hours from dawn to dusk to assure all birds had access to live food for the nestlings and fledglings. Granite grit and crushed oyster shell were provided ***ad libitum*** at 10 stations. Gravel placed on the soil surface of the potted trees and shrubs supplied additional gritting areas.

Sterile, shredded corn husk (The Andersons, Maumee, Ohio), and sterile coconut fiber (Animal Specialties Inc., Woodburn, Oregon) were provided for nest construction. Grass species were grown without pesticides in an on-site greenhouse and periodically harvested to supply additional nesting material. Nesting material was distributed to the floor and in shallow pans throughout the aviary. The birds also made use of leaves and needles from the shrubs and trees in the aviary.

Bird Identification and Group Assignment

To uniquely identify adult birds, a 2-mm x 12-mm barcode transponder (Avid Company, Norco, California) was implanted in the pectoralis muscle of each adult bird with a needle injector. Individual birds were identified when needed using a barcode reader (AVID Company, Norco, California). Constant recording barcode readers modified to record time signature with each barcode reading were installed around the nest box entrance hole to identify parental pairs and monitor nest activity (Figure 2). Nestlings were banded with a uniquely numbered leg band when they reached about 10 to12 days of age. Treatment group designation was made by colored leg bands (National Band and Tag, Newport, Kentucky). Clutches were randomly assigned to test groups by clutch period. Body weights of nestlings were taken on day 10 to 12.

Nest Boxes

A total of 115 nest boxes were placed in the aviary prior to the introduction of the sparrows to allow the birds to select boxes for nests. Some boxes were also used for night roosting during cold periods. To allow for easy removal to the exposure chambers, interlocking clips were used to hang the nest boxes at various heights (2 m to 3.5 m) throughout the aviary. Entrance holes of the nest boxes were oriented in the cardinal directions.

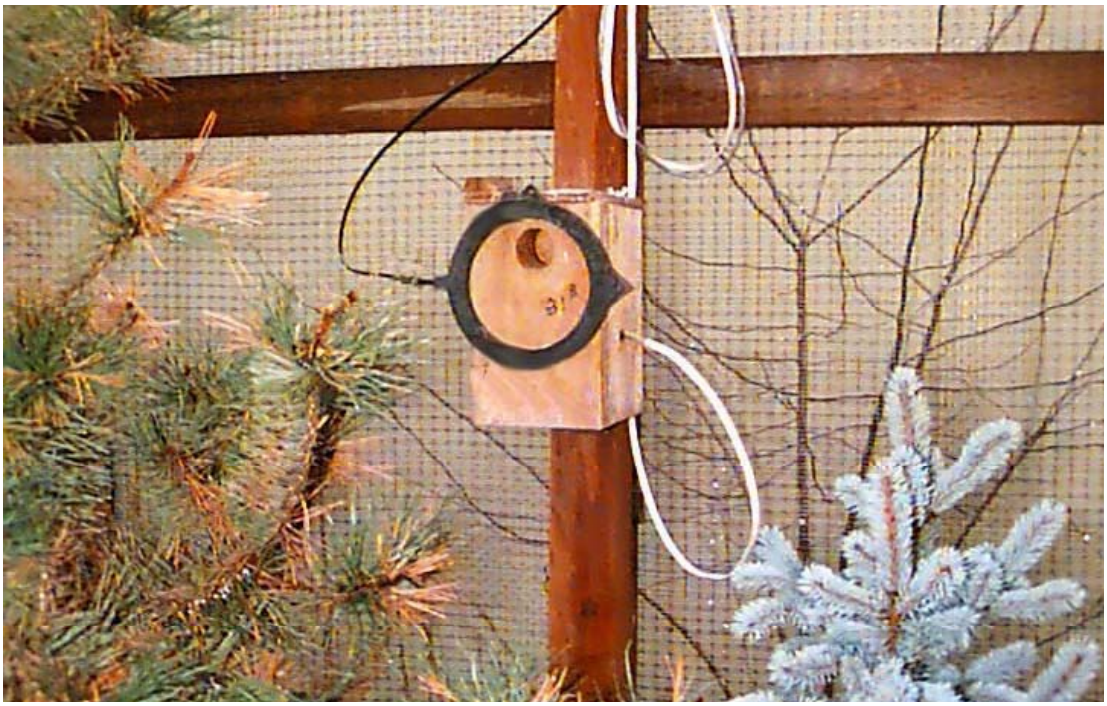

Figure 2. Nest box with barcode reader installed around the entrance hole to identify parental pairs and monitor nest activity.

Nest boxes were constructed of untreated rough cut quarter-sawn western red cedar (***Thuja plicata*** Donn) as described by Allen (1991). The boxes were 14 cm wide by 20 cm deep by 25 cm tall (6 in. x 8 in. x 10 in., Figure 3). A removable roof was added to the top of the box.

Internal dimensions of the nest boxes were modified from the straight core described in Allen (1991) to simulate more closely the conformation of naturally excavated cavities. Natural red-cockaded woodpecker nest cavities were obtained from wind-felled trees from Eglin Air Force Base, Niceville, Florida. The openings of these natural nest cavities (Figure 1) were measured to determine if the cavities had been occupied (and the openings enlarged) by woodpecker species other than the red-cockaded woodpecker. The entrance holes were 4.45 cm x 5.7 cm (1.75 in. x 2.25 in.) and were typical of red-cockaded woodpecker excavation. Therefore, the interior dimensions of the cavities were obtained by volume computed tomography (CT) imaging (Bio-Imaging Research, Inc., Lincolnshire, Illinois). A visualization program (3-D Studio VIZ, Kinetix®, Montreal, Quebec) was used to develop 3-dimensional models of the cavities from the internal 3-dimensional CT measurements of the cavities taken at 0°, 45°, and 90° at 1-in. intervals. The computer imaging composites of the interior structure of the cavities are shown in Figure 4; the dimensions obtained are shown in Figure 5. The artificially drilled cavities of the nest boxes were further excavated to more closely conform to the internal shape of the natural nest cavities.

Figure 3. Nest box as constructed for this project and fitted with sampling ports.

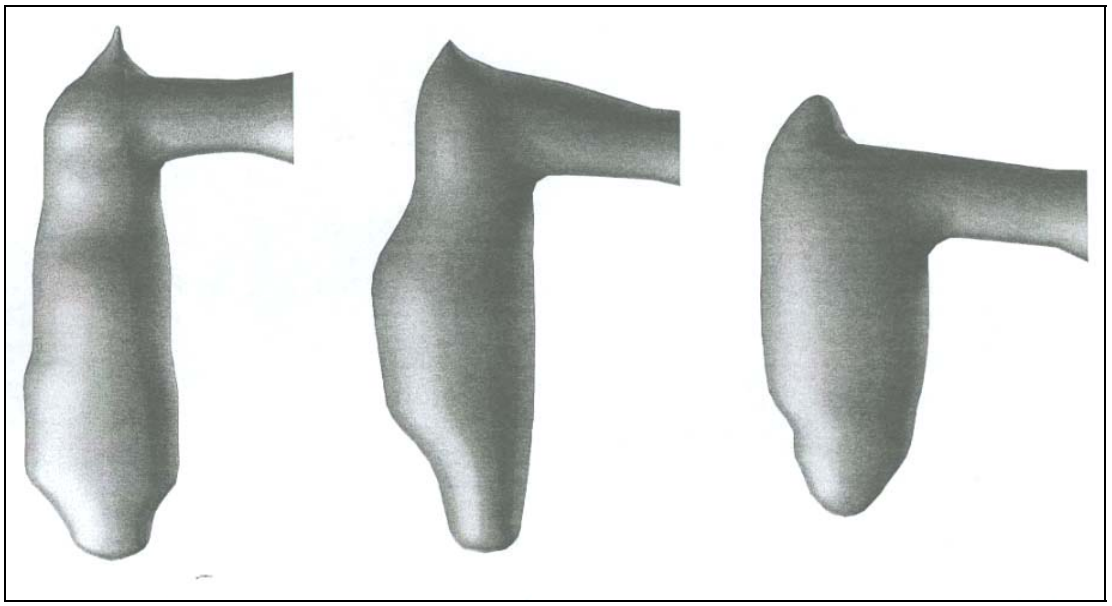

Figure 4. Three-dimensional models of 3 red-cockaded woodpecker nest cavities obtained from internal 3-dimensional X-ray volume computed tomography imaging and a 3-dimensional visualization tool.

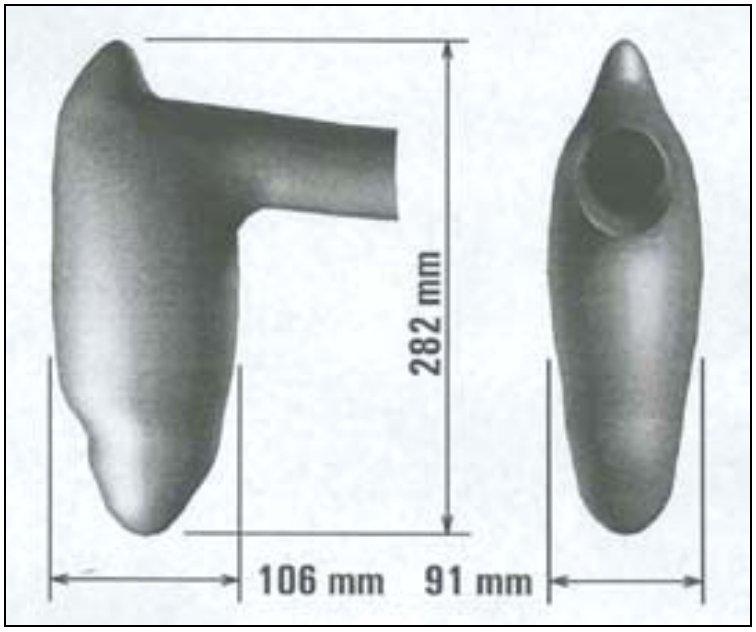

Figure 5. Internal dimensions were determined to excavate nest boxes to reflect the internal volume and shape of the natural cavities.

To compare the concentration profile inside the natural nest cavity with that of the artificial nest box, two holes were drilled into the lower portion of the cavities from the outside. Using the computer images of the internal cavities, a sampling port was drilled at the approximate breathing-zone height of the nestlings (5 cm above the floor) and a hole for returning air was drilled 2.5 cm higher and opposite from the sampling port hole. Stainless steel tubing 3.2 mm diameter penetrated about 1.3 cm into the cavity from each hole. The stainless steel tubing was extended to the outside of the exposure chamber or wind tunnel (see **Exposures** on page 23) by 3.2 mm diameter Teflon® tubing. The sampling line was connected to an Industrial Dust Sensor (IDS)-10 real-time aerosol monitor (MIE Inc., Bedford, Massachusetts) followed by a glass fiber filter, a sampling pump, and another filter. The air-returning line was connected to the exhaust of the sampling pump. A nest cavity was then placed on a rotating table in the environmentally controlled wind tunnel (Figure 6) at the Aerosol Research Laboratory at PNNL with the entrance hole near the center of the cross section of the wind. To simulate daytime temperatures and exposure conditions during the breeding season, the relative humidity in the wind tunnel was 40%, the temperature was 30°C (86°F), and the wind speed was maintained at 1-m/sec (2.2 mph). The entrance hole was sealed using an inflated weather balloon. Air was drawn from the cavity at a rate of 30mL/min to an IDS-10 real-time aerosol monitor and returned to the cavity (simulating the presence of an adult and/or young woodpeckers). Fog oil was then generated in the wind tunnel and monitored by a separate IDS-10 aerosol monitor. Filter samples were taken periodically to determine the actual mass concentration. Once the fog oil concentration stabilized

in the wind tunnel, the balloon seal was deflated and removed, allowing aerosol to enter the cavity. After the output voltage of the IDS-10 monitor for the nest cavity stabilized, a 13-mm filter sample and two OSH Versatile Samplers (OVS, SKC West Inc., Fullerton, California) samples were taken from the nest cavity to determine the aerosol concentration. A 47-mm and 13-mm filter sample were taken simultaneously from the wind tunnel and the nest cavity, respectively, to demonstrate there was no discrepancy using different sample flow rates. The flow rates were 60 mL/min for the 47-mm filter sample and 30 mL/min for the 13-mm filter sample. The aerosol concentrations determined from each filter were within 1 mg/m^3 of each other, indicating that the differing filter sizes and sample flow rates did not pose significant deviation in calculated aerosol concentrations.

Figure 6. Environmentally controlled wind tunnel at the Aerosol Research Laboratory at Pacific Northwest National Laboratory.

Profiles of FO concentration in a nest cavity and wind tunnel are shown in Figure 7. Measurements were taken with the nest cavity facing the airflow, perpendicular to the airflow, and facing away from the airflow to determine orientation for highest internal concentration. In general, the concentration profiles were similar regardless of the orientation (Figure 7).

FO deposition on eggs was estimated by placing Northern Bobwhite (***Colinus virginianus***) eggs in a nest box and exposing the box to FO as described for the test exposures. Eggs and foil deposition coupons were also placed on the floor within the chamber but outside the nest box. Foil deposition coupons were also placed on the nest box. Egg and foil samples were collected immediately after completion of the exposure, transferred to prepared sample bottles and refrigerated until analyzed.

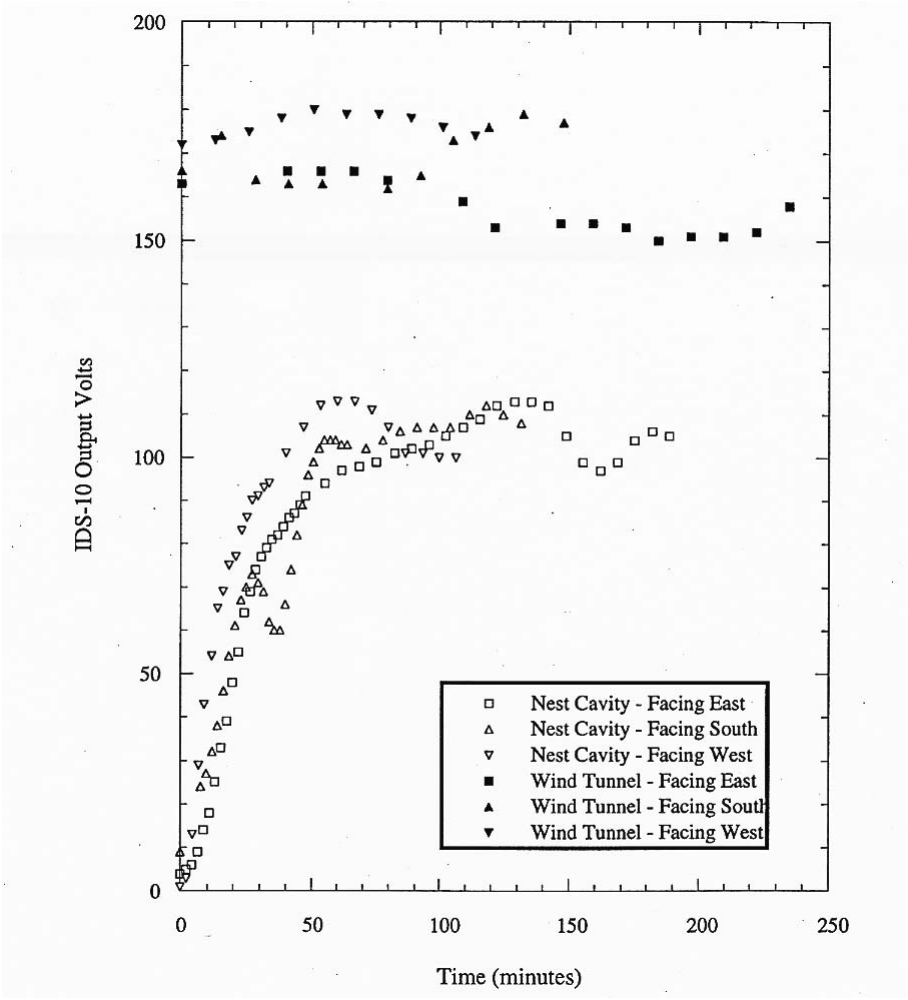

Figure 7. Aerosol concentration profiles in wind tunnel and nest cavity.

Exposure Chamber

FO concentration profiles in the nest cavities and wind tunnel showed that 30 to 50 minutes were required for the aerosol concentration to reach 90 percent of the stable concentration in the cavity. The time to reestablish the target exposure concentration of FO in the wind tunnel after introduction of a nest box was also long. To shorten the time eggs and nestlings would be separated from parental

care, a whole body exposure chamber (Sheet Metal Products, Young and Bertle Co, Cincinnati, Ohio, Figure 8) was used to provide a semi-dynamic FO exposure with a faster concentration recovery time. The concentration in the nest box cavities was the same as that found in natural nest cavities (about 75 percent of the external concentration) but reached the stable concentration in under 10 minutes (Figure 9). Therefore, the exposure chamber was used to deliver the test exposures.

Figure 8. This whole body exposure chamber was used to deliver fog oil test exposures.

Two inlet portals, one for introduction of fog oil aerosols and one for dilution air, were attached to the upper portions of the chamber. Two additional ports were installed in the chambers to obtain physical samples and allow a small flow to be withdrawn and passed to optical dust sensors for real-time monitoring of aerosol concentrations. A single exhaust port was used to control chamber vacuum, and directed aerosols to a wet scrubber/HEPA filtration system prior to venting to the outside. A vacuum gauge was fitted to the exposure chamber to aid in ensuring reproducibility of exposure conditions. The mean temperature and relative humidity during exposures were 31.0°C (30.5°C to 31.1°C) and 42.3 percent (41.7 to 44 percent RH), respectively.

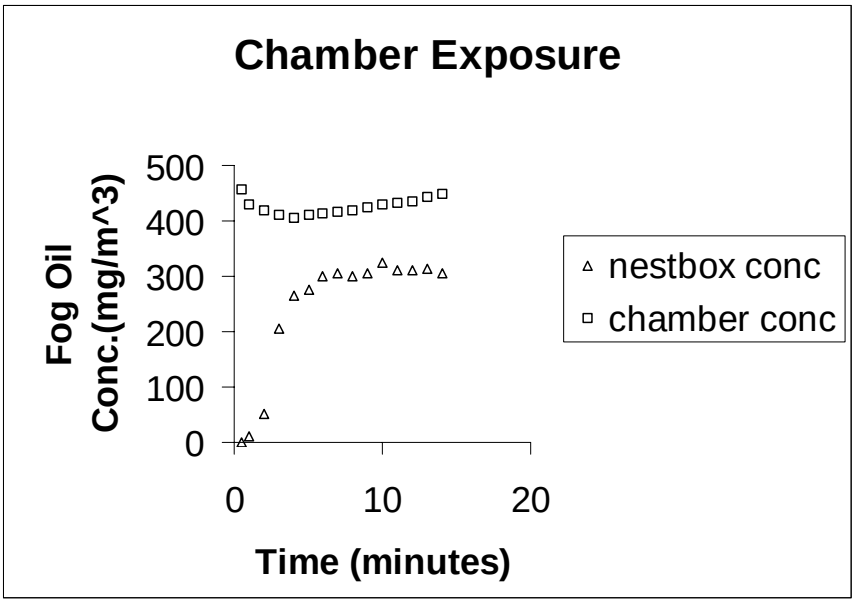

Figure 9. Concentration of fog oil in the nest box compared to concentration in the exposure chamber.

Test Material and Aerosol Generation

FO used in this study was Lot number 71808 manufactured by American Lubricating Company, Memphis, Tennessee, and supplied to PNNL by the U.S. Army National Training Center, Fort Irwin, California.

Aerosols were generated by metering steady rates of liquid FO onto a heated immersion element maintained at 600°C (Figure 10) and contained within a 1-m-long, 2.5 cm-diameter stainless steel pipe. The liquid fog oil was vaporized on the element and the vapor was subsequently recondensed as it cooled, forming a fog oil aerosol. Low-oxygen carrier gas (a mixture of 96 percent nitrogen and 4 percent air) was used to flush the condensing fog oil vapor through a temperature-controlled region at 300°C and into a 35-gallon (132-liter) buffer volume with a residence time of 5 minutes. The oxygen content of the carrier gas was about 0.8 percent, a value typical of the oxygen content present in the exhaust of diesel engines. In the buffer volume, fresh air was mixed with the concentrated fog oil aerosol and the mixture drawn through polyvinyl chloride (PVC) pipes into the exposure chamber or the wind tunnel at ambient temperature (18°C). A valve was used to adjust the flow of aerosol into exposure chamber or wind tunnel. A separate valve was used to regulate a flow of fresh air into the exposure chamber or wind tunnel.

To ensure mixing, restrictions were installed at the aerosol inlet to the chamber. The restrictions caused the fog oil aerosol to jet into the upper regions of the chamber and then quickly mix to a uniform concentration at the height of the entrance hole of the nest box or nest cavity. The feed rate of the oil was adjusted periodically, based on sensor-monitored aerosol concentration to maintain the test concentrations.

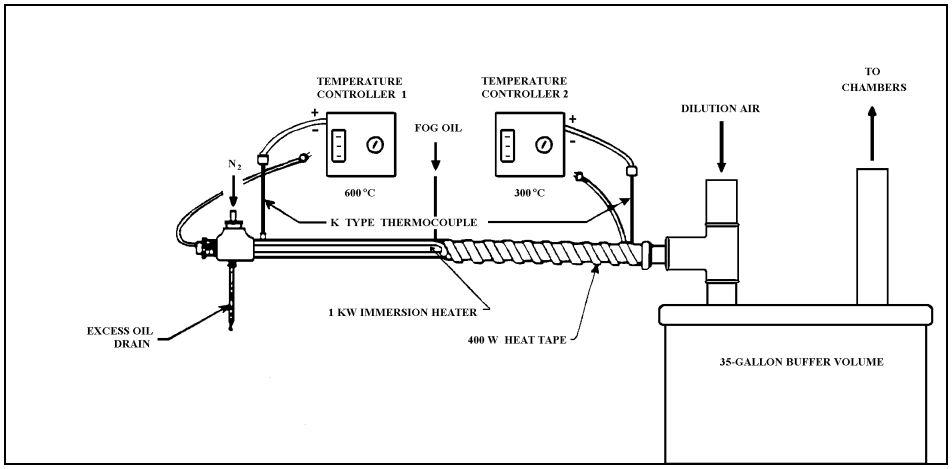

Figure 10. A schematic of the FO generator used to flash vaporize oil that is delivered to a buffer volume, then directed as an aerosol to the manifold and test chambers.

Fog Oil Characterization

Because the toxicity of petroleum oils is often related to the amount of PAHs present (Nam et al. 1999), samples of fog oil were collected prior to generation and during generation of FO smoke. Aerosol samples were collected during preliminary generations (airborne concentrations of 120 mg/m^3) on aluminum foil deposition coupons (929 cm^2). Samples were collected for 30 minutes. Unused foil coupons were used as blanks. All samples were placed in bottles with Teflon®-lined lids and stored at –20°C until analyzed for PAH content. Glassware and foil were ashed at 400°C for 24 hours and Teflon®-lined lids were rinsed with gas chromatography (GC) grade hexane and methylene chloride before use.

FO samples were extracted with methylene chloride according the National Oceanic and Atmospheric Administration's Status and Trends Program Technical Memorandum NMFS F/NWC-153 (Krahn et al. 1988). Samples were then cleaned using Silica/Alumina (5 percent deactivated) chromatography followed by High Pressure Liquid Chromatography (HPLC) cleanup. Selected deuterated surrogate PAH compounds were added at the beginning of each extraction to assess the efficiency of the method and all results corrected for the recoveries of the surrogates. Extracts were quantified using gas chromatography/mass spec-

trometry (GC/MS) in the selected ion mode (SIM) following a procedure based on U.S. Environmental Protection Agency (EPA) method 8270 (EPA 1986).

Foil and egg samples were extracted in their original containers with hexane. The oil samples were prepared by dilution of a known quantity of oil into hexane. All extracts were reduced to a final volume of 1 mL. PAH analyses were performed by a GC/MS single ion monitoring (SIM) method that is a modification of EPA method 8270. FO analyses were also performed by GC/MS SIM. This was accomplished by collecting ions representative of the fog oil hydrocarbon constituents and integrating and summing the entire area of the chromatogram due to the FO hydrocarbon response. Estimated detection limits were 25.2 ng/unit fog oil and about 10 ng/unit for PAH on the foil and eggs. PAH detection limits in the oil were about 500 ng/g. Method blanks were extracted with the samples and had concentrations less than the MDL (minimum detection limit) value. For the fog oil analysis, deuterated phenanthrene was used as a surrogate and for the PAH analyses, several deuterated PAH compounds were used as surrogates. These compounds were added to all samples prior to extraction to assess the efficiency of the method. These compounds were also used as internal standards because all data is corrected for the recovery of the surrogates. Surrogate recoveries were generally within the QC (quality control) limits of 40 to 120 percent. In both cases the sample concentrations were less than the MDL value. Blank spike had acceptable recoveries. Replicate precision was calculated based on the PAH results of the two FO samples.

Exposures

Clutches of eggs or broods of nestlings were exposed in their nest boxes during the first 5 days of their development. Nest boxes were transferred to the Aerosol Research Facility at Pacific Northwest National Laboratory (PNNL) within 5 minutes of arrival at the facility. Nest boxes were removed to an exposure chamber, rather than being exposed at the aviary, to eliminate variability in exposure concentration resulting from nest box location, presence or absence of parent, and poor control over exposure duration and uniformity of concentrations within large smoke clouds. Control nest boxes containing eggs or broods were similarly collected, transferred, and held in the exposure chambers but were not exposed to the aerosols. A group of active nest boxes with eggs or nestlings remained at the aviary and served as untreated aviary controls. Two concentrations of fog oil were used in the exposure tests and included a typical field concentration (100 mg/m^3) and a high, near-source concentration of 450 mg/m^3. Test concentrations were based on predictions from a modified Gaussian plume dispersion model for cogenerated aerosols of fog oil (Driver et al. 1993). The nest

boxes were exposed for 30 minutes at the treatment concentration. In addition, the nest boxes were exposed to 15 minutes of aerosols of lower concentration as the test atmospheres reached the target treatment concentrations.

After exposure, the nest boxes were returned to the outdoor aviary. Nest boxes were restored to original positions within 60 to 65 minutes of their removal. Post-exposure observations included number hatched, nestling mortality, number fledged, and potential signs of toxicity associated with the aerosol exposure. Parental behavior was observed for 30 minutes following return of the nest box, to confirm restoration of feeding and brooding activity of adults. Parental care was reestablished in every case. Fledglings were sacrificed and examined for gross and histologic lesions at 140 days post-exposure (approximating the parental dependence period for the red-cockaded woodpecker [Jackson 1987, 1994]). Ambient weather conditions during the acclimation and post-exposure periods were collected on site using a weather station consisting of an anemometer, a relative humidity sensor, a temperature sensor, a barometric pressure sensor, an 8-inch rain guage, and a datalogger (Met One Instruments, Inc., Grants Pass, Oregon).

Exposure Characterization

FO generations in the exposure chamber were monitored in real-time using an IDS-10 aerosol monitor. Filter samples were taken periodically to determine the actual mass concentration as described for the wind tunnel nest cavity exposures. Because the deposition velocity of particles to surfaces varies with the particle size, the aerodynamic diameter of the fog oil aerosol was determined using two Andersen ambient-style cascade impactors operated at 28 liters per minute (lpm). Temperature and relative humidity of the exposure chamber were measured during each test.

Statistics

Hatching and fledging success were converted to percentages for each clutch and then transformed with an arcsine transformation for binomial proportions (Zar 1974). The arcsine transformation was used because the range of percentages exceeded 30 to 70 percent. Bartlett's modification of the arcsine transformation for 0/n and n/n proportions was applied to clutches and broods producing 0 percent or 100 percent success (Zar 1974). A one-way ANOVA was performed on the transformed percentages. ANOVA was used to analyze clutch size and body weight of fledges. Statistical significance was determined by using an alpha level of 0.05.

3 Results and Discussion

Results

The maximum aerosol concentration in nest cavities under simulated field conditions was about 75 percent of the external air concentration (Figure 7). Filter samples of external and internal air were taken for three concentrations. At an external FO concentration of 57.5 mg/m^3, the air inside the nest cavity reached 42.5 mg/m^3 (73 percent). For external FO concentrations of 138 mg/m^3 and 425 mg/m^3, the internal FO concentrations were 100 mg/m^3 (73 percent) and 320 mg/m^3 (75 percent), respectively. Orientation of the entrance hole to the wind had little influence on cavity concentrations, though downwind orientation may come to steady state more rapidly (Figure 7). A similar concentration relationship between external and internal airborne FO was established for the nest boxes used for the exposure tests (Figure 9).

Chamber concentrations for the field-typical exposures averaged 98 ± 32 mg/m^3. The mean concentration of the high, "near-source" exposures was 490 ± 94 mg/m^3. The mass median aerodynamic diameter of the FO was 1.2 µm and the geometric standard deviation (GSD) was 1.7 during the test runs (Figure 11). This compares well with the MMAD of 1.2 µm ± 1.6 GSD measured during the red-cockaded nest cavity characterization tests in the wind tunnel and field values (Driver et al. 1993, National Research Council 1997).

FO deposition on quail eggs placed in the nest box was 70.6 ± 17.3 ng/egg. Deposition of FO to eggs in the open (outside the nest box) was not detectable on 22 eggs. Only two eggs had detectable concentrations of FO. The mean concentration for the 2 eggs was 28.4 ng/egg. After 30 minutes of exposure, the surface deposition of FO measured on foil coupons placed on the top of the nest box and at floor level was 5.8 ± 0.8 ng/cm^2 and 5.9 ± 3.9 ng/cm^2, respectively. No PAHs were detected in any of the egg or foil samples. The PAH composition of the stock fog oil prior to generation is shown in Table 1. No PAHs were detected in post-generation samples. Generation may not have been long enough to collect sufficient sample for PAH detection (but represents the amount to which the birds were exposed). In the previous study, compositional changes were observed during generation, producing an aerosol with greatly reduced levels of

napthalene (a highly toxic compound to birds) and minimal concentrations of higher weight PAHs (Driver et al. 2002).

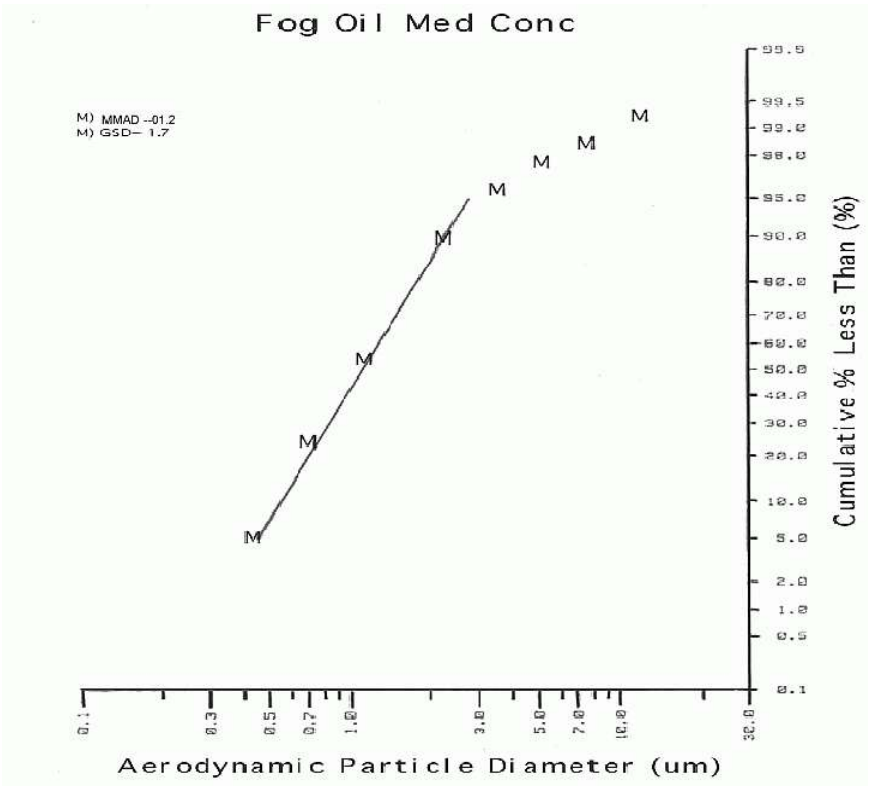

Figure 11. Particle size distribution and Mass Median Aerodynamic Diameter (MMAD) of fog oil aerosol in exposure chamber during test generations.

The number of clutches from each of three clutch initiating periods between March 29 and June 24 that were used in each treatment group is shown in Table 2. Nest success for each treatment group was calculated using only clutches with fertile eggs (Table 3). Of the 84 clutches produced during the study, only 4 contained infertile eggs. Three other clutches were lost, two due to deaths of parents and one from lack of incubation when the barcode reader slipped in front of the entrance hole excluding the parents. The number of eggs incubated per clutch was similar for all treatment groups (Table 3).

Table 1. Fog oil composition before and after generation.

	Conc (µg/g Fog Oil)	
Constituent	**Before Generation**	**After Generation[a]**
Lower Molecular Weight PAHs:		
Naphthalene	78.5	1.8
1-methyl naphtalene	98.3	<MDL[b]
Biphenyl	22.2	<MDL
2,6 dimethyl napthalene	110.0	<MDL
Acenaphthylene	2.4	2.5
Acenaphthene	3.5	3.2
2,3,5 trimethyl napthalene	70.7	<MDL
Fluorene	19.1	21.5
Dibenzothiophene	63.9	264.0
Phenanthrene	40.8	162.0
Anthracene	3.6	15.2
1 methyl phenanthrene	49.2	<MDL
Fluoranthene	2.2	27.2
Higher Molecular Weight PAHs:		
Pyrene	14.7	40.2
Benzo(a)anthracene	1.8	14.5
Chrysene	9.3	57.2
Benzo(b)fluoranthene	2.5	MDL
Benz(k)fluoranthene	<MDL	<MDL
Benzo(e)pyrene	2.2	<MDL
Benzo(a)pyrene	0.5	<MDL
Perylene	1.1	<MDL
Indeno(123-cd)pyrene	<MDL	<MDL
Dibenzo(a,h)anthrancene	<MDL	<MDL
Benzo(g,h,i)perylene	0.6	<MDL

(a) No PAHs were observed in the generated aerosol during the exposure sampling. Post-generation values from a previous study (Driver et al. 2002) in which 2- and 3-hour collections were made are reported.

(b) Less than the minimum detection limit.
Recovery of Surrogates: d8 Naphthalene 83.9%; d10 Acenaphthene 79.0%; d10 Phenanthrene 78.9%; d12 Chrysene 60.6%; d12 Perylene 95.3%; d14 Dibenzo[a,h]anthracene 110%.

Table 2. Number of clutches used in study by clutch period.

	Clutch Order			
Treatment	**1st**	**2nd**	**3rd**	**Total**
Aviary Control	10	5	1	16
	(10)	(6) [a]	(3) [b]	(20)
Test Control	9	6	5	20
	(10) [c]	(6)	(5)	(21)
100 mg/m^3 FO	10	6	5	21
	(10)	(6)	(5)	(21)
450 mg/m^3 FO	8	7	5	20
	(9) [a]	(7)	(6) [a]	(22)
Values are number of clutches used for calculations of reproductive success. (Values in parentheses are number of clutches treated.)				
(a) Infertile eggs.				
(b) 2 nests with dead adult, eggs unhatched				
(c) Clutch lost when barcode reader blocked entrance.				

Table 3. Hatching and fledging success of house sparrow clutches exposed to fog oil aerosols.

		Fog Oil (mg/m^3)		
Variable	**Aviary Control**	**0**	**100**	**450**
Number of clutches	16	13	13	12
Mean number of eggs/clutch	3.2(0.28)	2.9(0.31)	3.1(0.19)	2.8(0.26)
Mean number of nestlings/clutch	2.0(0.35)	1.4(0.28)	1.7(0.33)	1.1(0.31)
Mean number of fledglings/clutch	1.8(0.35)	1.2(0.28)	1.5(0.31)	1.0(0.29)
%Hatched[(a)]	59.0	52.8	56.6	51.7
% Fledged[(b)]	50.5	46.5	41.2	46.3
Values are means (SE).				
(a) Percentage of birds that hatched is based on the number hatched/number of eggs in the clutch X 100. No significant differences among means was found, ANOVA of arcsine transformed ratios of hatched (P=0.38). Values are percentages corresponding to arcsine transformations.				
(b) Percentage of birds that fledged is based on the number fledged/number of eggs in the brood X 100. No significant difference between control and treatment fledging success, ANOVA of arcsine transformed percent fledged (P=0.81). Values are percentages corresponding to arcsine transformations.				

Hatching success was not significantly different between treatment groups (Table 3) and was comparable to values (52.1 to 64 percent) observed for house sparrow nests built in both tree and nest boxes (McGillivray 1983). The fledging success of nestlings from nest boxes exposed to FO aerosols was not different from the success of control clutches (Table 4). Exposure of developing nestlings to FO also did not result in significant differences in fledging success.

Table 4. Fledging success of house sparrow broods exposed to fog oil aerosols.

		Fog Oil (mg/m3)		
Variable	**Aviary Control**	**0**	**100**	**450**
Number of broods	11	7	8	8
Mean number of nestlings/brood	2.5(0.36)	3.1(0.33)	2.4(0.32)	2.6(0.46)
Mean number of fledglings/brood	2.2(0.31)	2.3(0.37)	2.0(0.28)	2.3(0.50)
%Fledged[(a)]	81.9	74.2	78.7	80.0
Values are means (SE).				
(a) Percentage of birds that fledged is based on the number fledged/number of hatchlings in the brood X 100. No significant differences among means; ANOVA of arcsine transformed ratios of fledged (P=0.66) to number of eggs in clutch. Values are percentages corresponding to arcsine transformations.				

Fledgling survival to 140 days was not different among treatment groups (Table 5). Many of the fledgling deaths occurred during a 2-week period of rain and near freezing weather when fledges were newly out of the nest. Most were underweight at death. A larger number of the broods among the aviary controls fledged during this period and may be reflected in the slightly elevated mortality rate and reduced body weight of the dead fledglings from this group (Table 5). Six of the fledglings that died had moderate to severe granulomatous hepatitis associated with protozoan parasitism (***Pasmodium*** sp.) The incidence of parasitism was broadly distributed among control and treatment groups and was unrelated to treatment. One control fledgling exhibited marked extramedullary hematopoiesis (EMH) in the liver. In an older bird this would likely indicate hematologic disorders, but EMH may be normal in younger birds of this species.

Table 5. Post-fledge mortality.

		Fog Oil (mg/m^3)		
Variable	**Aviary Control**	**0**	**100**	**450**
Number fledglings	25	34	37	31
Number Died Post-Fledge				
Exposed in ovo	NA	2	6	2
Exposed as nestling	NA	6	2	5
Total	7	8	8	5
Number with parasitic lesions	1	2	2	1
%Mortality[(a)]	28.0	23.5	21.6	22.5
Post-mortem body weight (g) [(b)]	23.6 (2.2)	24.9 (3.4)	26.5 (3.6)	25.4 2.3
(a) Post-fledge mortality includes juvenile birds that died up to 140 days after leaving the nest.				
(b) Values are means; values in parentheses are one standard deviation of the mean.				

Discussion

Because the mass concentration of suspended droplets in the nest boxes is the characteristic most directly linked to the potential amount of fog oil inhaled by nestlings or deposited on the nestlings and eggs, it is essential to hazard assessments that surrogate exposures closely match those experienced by their endangered counterparts. The nest boxes in this study were modified to conform to the excavations of the red-cockaded woodpecker and obtained internal concentrations similar to those observed in the natural woodpecker cavities. Concentrations appear to stabilize at about 75 percent of the external concentrations in both the nest boxes and natural cavities. This is consistent with the results of a related study (Guelta and Checkai 2001) that examined the degree to which cavities reflected ambient concentrations of fog oil under different conditions of wind movement and cavity orientation. In that study, fog oil concentration within the cavity ranged from 61 to 94 percent of the atmospheric levels for one series of tests, and averaged 84 to 95 percent in a second series. Interestingly, the deposition of fog oil to eggs is much greater (albeit very low) in the nest box than in the open. This may result from gravitational settling of retained droplets. FO in the exposure chamber is purged prior to retrieving the nest boxes, thus reducing post-exposure settling. This scenario may be similar to exposures in the field with wind dissipating the FO oil externally while FO in the nest cavities is retained. However, FO aerosols within the nest cavity would probably be purged by air movement caused by parental activity.

Particle size distribution of FO aerosols also influences the transfer rate to the internal cavity of the nest boxes and deposition on eggs and nestlings and within the respiratory tract of the nestlings. FO aerosols in the test exposures had droplet size characteristics similar to those generated in the field (Driver et al. 1993) assuring a realistic exposure simulation.

No decrease in hatchability of sparrow eggs from nest boxes exposed to up to 450 mg/m^3 of FO for 30 minutes was observed in this study. FO concentration within the nest boxes was shown to reach about 75 percent of the external concentration (i.e., over 300 mg/m^3). Although there was greater deposition of FO to the eggs inside the nest boxes than to eggs placed in the open, the amount deposited to the egg surfaces was small (about 70 ng/egg). Yet, there are numerous reports of significant embryotoxicity resulting from application of minute amounts of crude and refined petroleum to egg surfaces (White et al. 1979, Albers 1977, Couillard and Leighton 1990, Trivelpiece et al. 1984, Macko and King 1980, Albers 1980). Large reductions in hatching success were seen whether the eggs were artificially or naturally incubated (White et al. 1979, Macko and King 1980). It may be that FO is less toxic to developing embryos than other lubricating oils or that

deposition of FO oil to the eggs was below the toxic threshold. As little as 1 microliter (920 µg) of crude oil was shown to increase mortality in chicken eggs (Couillard and Leighton 1990), but this application exceeds, by a factor of 10,000, the amount of FO aerosol deposited to eggs in the nest boxes. Aerosol application may also diminish the potential hazard of FO deposition to developing eggs. In studies where mallard (***Anas platyrhynchos***) eggs were sprayed with an aerosol of No. 2 fuel oil, hatchability was less affected by aerosol deposits than by equivalent amounts of oil deposited by microliter syringe (Albers and Heinz 1983). Presumably, deposition of the dose over a much greater surface area resulted in the lessened impact. The likely smaller droplet size of the FO aerosol would probably reduce the impact even further. In any case, military use of the aerosol appears to be unrelated to direct, heavy application of oil to either eggs or nestlings.

Overall clutch success as measured by the percentage fledged and survivability to 140 days was not adversely affected by FO exposure and was similar to values reported for wild house sparrows nesting in both trees and nest boxes (McGillivray 1983, Hegner and Wingfield 1987). Mortality of fledges appeared to be attributable largely to weather and parasitism and was unrelated to FO exposure.

4 Conclusions

FO exposure up to 450 mg/m^3 for 30 minutes during sensitive periods of embryonic and nestling development did not adversely impact hatchability of house sparrow eggs or the fledgling success and survivability of sparrow young. The exposure to airborne FO is somewhat reduced (about 25 percent) within red-cockaded woodpecker nest cavities and similarly constructed nest boxes. However, deposition on eggs and other surfaces during periods of low parental activity may be enhanced inside excavated nests. It is unlikely that such accumulations, even over repeated exposures, will result in reduced nest success because the deposition rate is low. Also, the relatively broad deposition area of aerosol applications appears to greatly reduce the embryotoxic hazard of lubricating oils. It should be noted that the embryotoxicity of FO has not been determined. Normal military use of fog oil smoke, therefore, does not appear to be hazardous to altricial birds, such as the red-cockaded woodpecker, at the egg or nestling stages of development.

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Acronyms

ANOVA	analysis of variance
CERL	Construction Engineering Research Laboratory
CT	computed tomography
EMH	extramedullary hematopoiesis
EPA	U.S. Environmental Protection Agency
ERDC	Engineer Research and Development Center
ESMP	endangered species management plan
FO	fog oil
GC	gas chromatography
GC/MS	gas chromatography/mass spectrometry
GSD	geometric standard deviation
HPLC	high pressure liquid chromatography
INRMP	integrated natural resources management plan
MDL	minimum detection limit
MMAD	mass median aerodynamic diameter
PAH	polynuclear aromatic hydrocarbon
PNNL	Pacific Northwest National Laboratory
PVC	polyvinyl chloride
QC	quality control
RCW	red-cockaded woodpecker
SERDP	Strategic Environmental Research and Development Program
SGF-2	liquid fog oil formulation used in these studies
SIM	selected ion mode; single ion monitoring

CERL Distribution

Chief of Engineers
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SERDP (3)

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE (DD-MM-YYYY)
12-2002

2. REPORT TYPE
Final

3. DATES COVERED (From - To)

4. TITLE AND SUBTITLE
Effects of Fog Oil Smoke on the Hatchability and Fledgling Survival of the House Sparrow (Passer domesticus), a Nestling Surrogate for the Red-cockaded Woodpecker

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER
SERDP

5e. TASK NUMBER
CS-507

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
U.S. Army Engineer Research and Development Center (ERDC)
Construction Engineering Research Laboratory (CERL)
PO Box 9005
Champaign, IL 61826-9005

8. PERFORMING ORGANIZATION REPORT NUMBER
ERDC/CERL TR-02-34

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Strategic Environmental Research and Development Program
901 N. Stuart St., Suite 303
Arlington, VA 22203-1821

10. SPONSOR/MONITOR'S ACRONYM(S)
Program Manager

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES
Copies are available from the National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161.

14. ABSTRACT

Eggs and nestlings of captive house sparrows (*Passer domesticus*) were exposed for 30 minutes to either field typical (100 mg/m^3) or higher (450 mg/m^3) concentrations of fog oil aerosols. Nest boxes were excavated according to the internal dimensions of natural cavities of the red-cockaded woodpecker (*Picoides borealis*) and the fog oil concentration within the artificial and natural cavities compared to ensure exposure realism. Internal concentrations stabilized at about 75 percent of the external airborne concentration of fog oil. Deposition of fog oil on eggs was measured and found to be low but greater than found on eggs placed outside the nest boxes. Although exposures were made during sensitive development periods (first 5 days of embryonic or nestling growth), no adverse effect on hatchability, fledgling production and survivability were observed.

15. SUBJECT TERMS

house sparrow	fog oil	environmental impact
Red-cockaded Woodpecker	smoke and obscurants	endangered species birds

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			Harold Balbach
Unclassified	Unclassified	Unclassified	SAR	38	**19b. TELEPHONE NUMBER (include area code)** 217-352-6511, ext 6785